iGEM 2016: Team Pittsburgh
Week 20 Lab Notebook

Monday, October 3

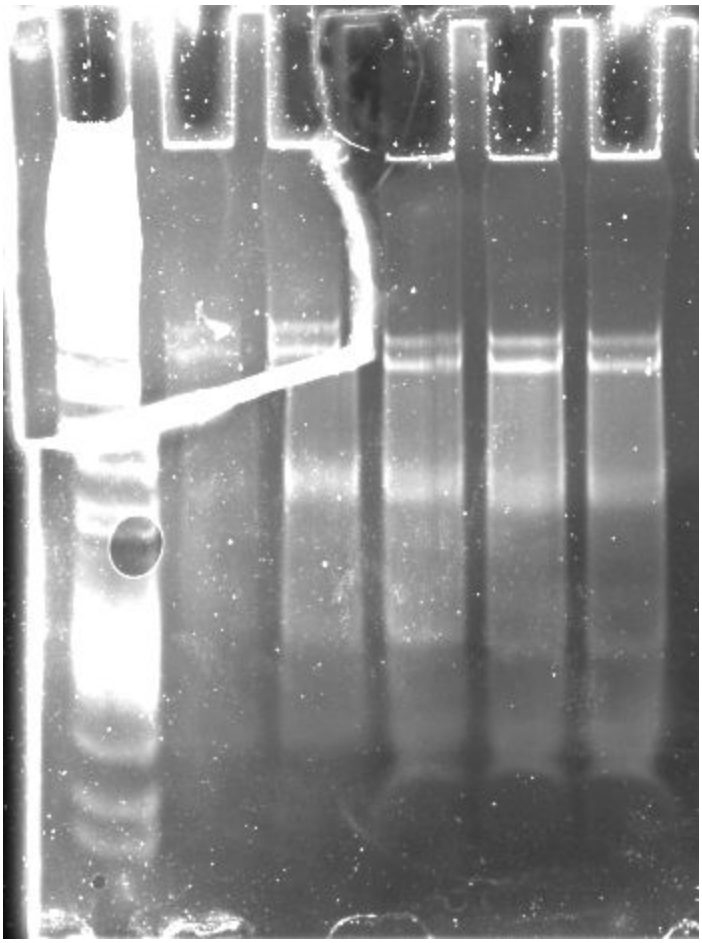
Cleavage reactions (Claire)

50 nM renatured DNAzyme	2.5 uL 1 uM DNAzyme
2 uM lead	2.5 uL 40 uM lead
Buffer (3) or water	45 uL
	50 uL

Incubate at 37 for 105 min

Tuesday, October 4

PAGE analysis (Praneeth)



Wednesday, October 5

Maddie pcr

Friday, October 7

Check PT3-GFP and PT7-T3 (Claire)

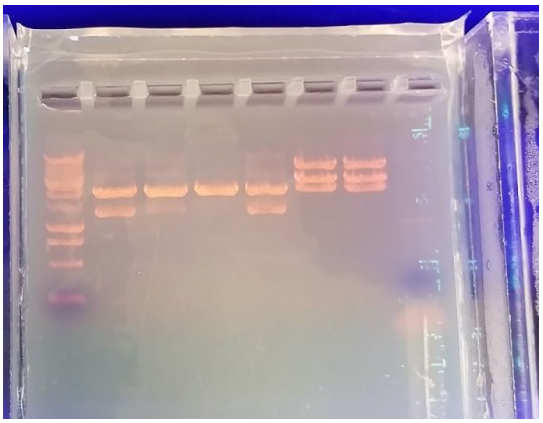
Digest:

0.5 uL EcoRI
0.5 uL PstI
1 uL buffer
1 ug plasmid
10 uL with water

DpnI digest of lacZ (Claire)

Incubate 1 hr 30 min at 37

1% agarose gel, 100 V, 37 min (Claire)



Lane 1 ladder
2 PT7-T3 1
3 PT7-T3 2
4 PT7-T3 4
5 PT7-T3 5
6 PT3-GFP 3:1
7 PT3-GFP 7:1
8 DpnI digested lacZ

Extract lacZ (Claire)

Gel slice didn't fully dissolve. Added another aliquot of extraction buffer to undissolved gel after liquid was transferred to column (ethanol had been added). lacZ 2 is second go

Concentrations: lacZ 1: 0.8 ng/uL
lacZ 2: -0.3 ng/uL

Ligation (Aife)

Add 2 uL T4 DNA ligase buffer
7 uL water
1 uL T4 DNA ligase

Transformation (Aife)

Entire reaction into Cheryl's cells

Saturday, October 8

Cell-free lead titration

10/7

Saturday, October 7

Cell-free ~~DNA~~ lead titration & mismatch

25 ng switch, 3.74 nM hp

Control: G, ~~3~~ 2 μM lead.

Conditions: ~~D, 2 μM lead~~

G, ~~4~~ 50 nM lead

72 nM lead

100 nM

~~250~~ 500 nM

~~2~~ 1 μM

25 μM

50 μM

no trigger, G

~~50~~ 3.74 nM DNA

hairpin only

~~10 RXNS~~ conditions, 22 rxns

no ~~trigger~~, ~~G~~ D

D + 3.74 nM ~~G~~ DNA

D + hairpin only

D + 3.74 nM D ~~DNA~~

Sol'n A	~~2~~	2.4	~~36~~	72
B	~~1.5~~	1.8	27	54
RNAse	~~.2~~	0.3	~~4.5~~	9
substrate		0.3	4.5	9
trigger		0.6	~~0~~	18
switch		.31	~~9~~	9.46
H_2O		0.29 } 0.6		8.54 } 18

~~30 rxns~~ ~~x15 rxns~~

10/7

2 / 1.448 = 1.38 ; 1 / 10 / 3 / .38 3.8 1.14

TRIGGERS: set up Friday night & freeze

A1-2 no trigger — 1.2 µL H_2O

3-4 hairpin only, 3.74 nM — 1 µL hp @ 74.8 nM + 1 µL H_2O] 1.2 µL hp @ ~~37.4~~ 37.4

~~3.74 nM hp + 50 nM lead — 1 µL hp @ 74.8 nM + 1 µL Pb^{+2} @ 1 µM] 1.2 µL~~

6 EPA MCL — 3.74 nM hp + 72.4 nM lead — 1 µL hp @ 74.8 nM + 1 µL Pb^{+2} @ 1.448 µM] 1.2 µL

for mismatch experiment (10/10) — ~~+ 100 nM Pb^{+2} — 1 µL hp @ 74.8 nM + 1 µL Pb^{+2} @ 2 µM] 1.2 µL~~

7-8 + 250 nM Pb^{+2} — 1 µL hp @ 74.8 nM + 1 µL Pb^{+2} @ 5 µM] 1.2 µL

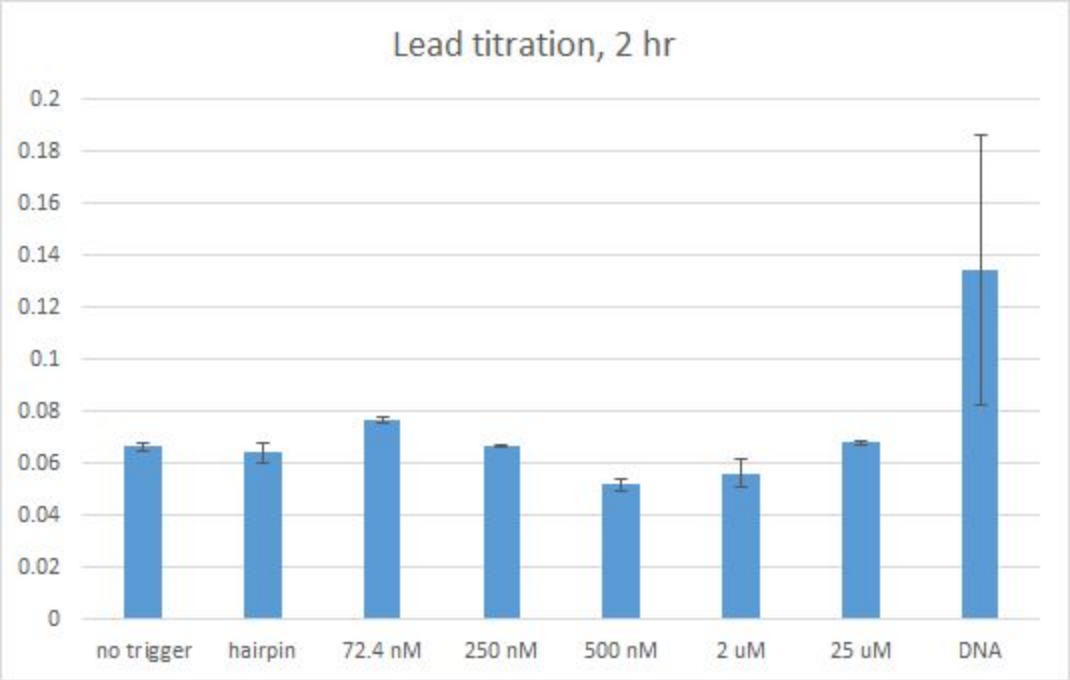
9-10 + 500 nM Pb^{+2} — 1 µL hp @ 74.8 nM + 1 µL Pb^{+2} @ 10 µM] 1.2 µL

11-12 + 2 µM Pb^{+2} — 3 µL hp @ 74.8 + 3 µL Pb @ 40 µM] 1.2 µL

13-14 + 25 µM Pb^{+2} — 1 µL hp @ 74.8 nM + 1 µL Pb @ 500 µM] 1.2 µL

~~+ 50 µM Pb^{+2} — 1 µL hp @ 74.8 nM + 1 µL Pb @ 1 mM] 1.2 µL~~

15-16 3.74 nM DNA — 1.2 µL DNA @ 37.4 nM



Everything still yellow--should have let reaction go for a bit longer